Research Note

Two common European viviparid species hybridize

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Abstract: During a morphological/electrophoretic study on the European Viviparidae, a hybrid but fertile female specimen [*Viviparus contectus* (Millet, 1813) x *V. viviparus* (Linnaeus, 1758)] was found in the Niepolomice Forest, South Poland. The specimen showed intermediate characters in its shell, anatomy, and embryonic shell. Polyacrylamide gel electrophoresis of that specimen confirmed its hybrid origin, which was marked in eight enzyme systems: specific homozygotes in both species along with a typical heterozygote in the hybrid. Interspecific differences in allozyme pattern, with different alleles in at least one locus in most enzyme systems studied, seem to indicate that *V. contectus* and *V. viviparus* are very old species, and their isolating mechanisms preventing hybridization could have become not efficient enough after such a long time since the speciation event. This is all the more probable, now that they occur sympatrically very rarely.

Viviparus contectus (Millet, 1813) and *V. viviparus* (Linnaeus, 1758) are widely distributed and common in Central Europe. However, their diagnostic characters are labile (Falniowski, 1989, 1990). In particular, this concerns shell characters, in which the presence of the umbilicus (which can be partly covered but is always visible) in *V. contectus* contrary to its absence in *V. viviparus* seems the most constant difference. Falniowski (1990: Plate iii, phot. 16-19) presented photographs of shell intermediates of the two species. On the other hand, those intermediates were undoubtedly determined anatomically as *V. contectus*. The only anatomical difference between the two species has been found in the female reproductive organs (Falniowski, 1989, figs. 45-52, also Falniowski, 1990, fig. 362A, B). In *V. contectus* the duct of the receptaculum seminis is usually narrow while leaving the receptaculum, whereas in *V. viviparus* it is strongly (rather strongly) widened; at the duct section parallel to the receptaculum, in *V. contectus* there commonly (not always) is a bulbous widening, which is absent in *V. viviparus*.

In the literature, one can find numerous opinions on ecological vicariance of the two species (e.g. Zhadin, 1928). In fact, the distribution pattern of the two species in Poland is slightly different from the one described by Zhadin (1928), but the species rarely occur sympatrically (Falniowski, 1989).

In 1976, a giant female of *Viviparus* (Fig. 1) was found in Drwinka River in Niepolomice Forest (about 30 km E from Kraków, Southern Poland). The shell measured 55 x 42 mm (normally, in *V. contectus* and *V. viviparus* it does not exceed 45 x 36 mm and 40 x 28 mm, respectively). The specimen was identified (based on shell characters alone; anatomical differences had not been known until then) as *V. contectus f. hungaricus* Hazay, 1881 (Falniowski, 1989). The shell, although more similar to *V. contectus* than to *V. viviparus*, was certainly not typical of *V. contectus*, and resembled the shell of the Danube *V. acerosus* (Bourguignat, 1862).

The weakness of diagnostic characters within the family Viviparidae is as already mentioned above, and relationships and taxonomy within the group are poorly understood. The species are described solely upon a basis of shell characters and no further study has confirmed their distinctness, although viviparids are often used as laboratory animals in various studies. Such are the reasons for starting morphological and electrophoretic studies (Table 1) on the European representatives of the family. Until now, we have already found constant differences in numerous enzyme systems among *Viviparus contectus, V. viviparus* and *V. acerosus* (Table 2), coupled with an extremely low proportion of polymorphic loci in the three species. The study is in progress.

Among viviparids collected from a small pond at Ispina, the Niepolomice Forest, in August 1991, we found one big specimen, the shell of which resembled the one of *Viviparus acerosus*, as well as the one collected in 1976 (Fig. 1) in the Drwinka River (the same region, one locality a couple of kilometres from the other). The pond is situated between the Vistula River and its right-bank dyke that is the north border of the Niepolomice Forest, Wislisko-Kobyle

Table 1. Application of polyacrylamide gel electrophoresis technique.

Electrophoresis: in slabs (180 x 130 x 0.7 mm) of 7.5% polyacrylamide gel in a continuous high-pH buffer system of Davis (1964).

Reservoir buffer: Tris-glycine (pH 8.3); 3 g Tris and 1.4. glycine per 1 *l* water.

Stacking gel buffer: Tris-HCl (pH 6.8); 6 g Tris titrated to pH 6.8 with 1M HCl in 100 ml final volume.

Resolving buffer: Tris-HCl (pH 8.8); 36.3 g Tris and 48 ml 1M HCl mixed and diluted to 100 ml final volume.

Acrylamide-bisacrylamide solution: 30 g acrylamide and 0.8 g bisacrylamide diluted to 100 ml final volume and filtered.

Stacking gel: 2.5 ml acrylamide-bisacrylamide solution, 5 ml stacking gel buffer, 2.5 ml 0.004% riboflavin, 10 ml water and 0.015 TEMED mixed and photopolymerized.

Resolving gel: (7.5%): 15 ml acrylamide-bisacrylamide solution, 7.5 ml resolving gel buffer, 39.5 ml water and 0.03 ml TEMED polymerized with 3 ml 1.5% ammonium persulfate as the catalyst.

Rns: Fourteen samples, 20 µl each, applied for a slab. Typically, a current 20-30 mA maintained for about 4 hrs until a marker dye (bromophenol blue) passed all the slab.

Reserve. The pond is about 200 m long and 50 m wide; its depth does not exceed about 2 m; there is water in the pond all year round. The shores are sandy, while the deeper parts are muddy. Along the shores, some scarce aquatic plants can be found. Besides the acerosus-like specimen, only *V. viviparus* were found occurring in mass along the shore.

There is another, small pond, situated about 500 m down the river from the one described above. Its distance from the dyke is the same as that of the former pond, but its length and width are smaller by half. Its character is also quite different: the depth is less than 1 m; the vegetation is rich, and there is no mud. The small pond temporarily desiccates. It is inhabited by a dense population of *Viviparus contectus*. Although the shells of *V. contectus* at this site are small and show some characters, in which they resemble *V. viviparus*, they are typical *V. contectus*, both anatomically and electrophoretically. The two ponds are temporarily linked by floods, which makes gene flow possible.

The big, acerosus-like specimen had the shell 44.0 mm high and 33.8 mm broad. The umbilicus was a cross between the ones of *Viviparus contectus* and *V. viviparus*, while the shell colouration with distinct bands resembled rather the latter species. Also the operculum showed intermediate characters. The specimen was a female. The receptaculum seminis with its duct (Fig. 2) resembled more the one typical of *V. viviparus*, but its diagnostic characters were intermediate enough to make determination hardly possible. There were as many as 32 embryos in the brood pouch, which is a number typical of the Polish viviparids, especially of *V. contectus* (Falniowski, 1989). From among the embryos, 17 were in early stages, with no shell; eight had small shells; seven were ready to be born. The maximum shell dimension of the embryos in the latter group was 4.4 - 5.0 mm; the shell height was up to 4.2 mm. The shells had a covered umbilicus (like in *V. viviparus*), and a blunt apex (but sharper than in *V. viviparus*). There were two rows of vestigial bristles along the body whorl. Similar

Table 2. Enzyme systems showing constant differences (no allele in common in at least one locus) between *Viviparus contectus* and *V. viviparus*, and typical heterozygotic bands in the hybrid specimen.

Enzyme name	Enzyme E.C. number	staining after
1. ß-hydroxybutyrate dehydrogenase	E.C. 1.1.1.30	Wurzinger (1979)
2. Phosphoglucomutase	E.C. 2.7.5.1	Wurzinger (1979)
3. Leucine aminopeptidase	E.C. 3.4.11.1	Rudolph and Burch (1987)
4. Lactate dehydrogenase	E.C. 1.1.1.27	Richardson *et al.* (1986)
5. Malate dehydrogenase	E.C. 1.1.1.37	Wurzinger (1979)
6. Phosphohexose isomerase	E.C. 5.3.1.9	Wurzinger (1979)
7. Hexokinase	E.C. 2.7.1.1	Richardson *et al.* (1986)
8. Adenylate kinase	E.C. 2.7.4.3	Richardson *et al.* (1986)

Fig. 1. *Viviparus contectus f. hungaricus* Hazay, 1881, from the Drwinka River, Niepolomice Forest (after Falniowski, 1989, plate II, phot. 9), specimen in the collection of the Zoological Museum of Jagiellonian University, Kraków. MZUJF00217.

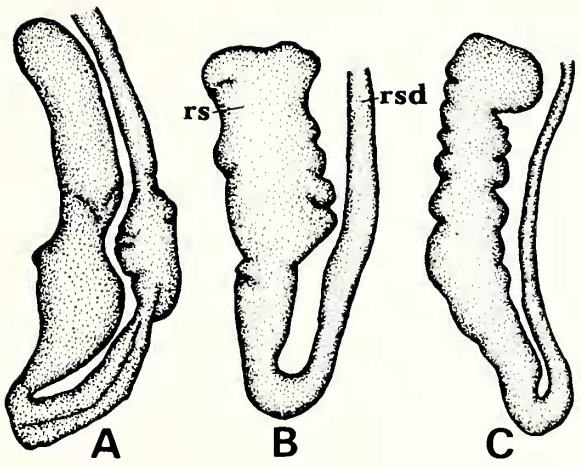

Fig. 2. Diagnostic characters in viviparid female reproductive system: receptaculum seminis (rs) with its duct (rsd): A, *Viviparus contectus;* B, the hybrid specimen; C, *Viviparus viviparus.*

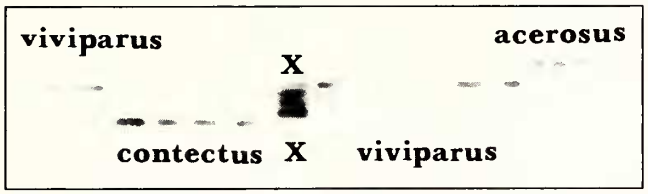

Fig. 3. Zymogram of ß-hydroxybutyrate dehydrogenase of *Viviparus viviparus, V. contectus, V. acerosus* and the hybrid specimen (marked with two "X").

bristles but longer are characteristic of young *V. contectus*, whereas young *V. viviparus* have no bristles at all.

Polyacrylamide gel electrophoresis (Table 1) of the hepato-pancreas tissue of this curious specimen revealed its hybrid character (*Viviparius contectus* x *V. viviparus*), which was detected at eight loci of eight different enzymes (Table 2, Figs. 3, 4). At the same time, no similarity in allozyme pattern was found between the specimen and *V. acerosus.* ß-hydroxybutyrate dehydrogenase (Fig. 3) provides a good example, with specific homozygotes in each species, and a typical heterozygote in the hybrid specimen.

The above data look interesting, but their more profound interpretation requires a better understanding of viviparid relationships. It seems that *Viviparus viviparus* and *V. contectus* can produce fertile hybrids in natural conditions. Such hybrids are not common: among about 150 specimens collected at the same locality in May 1992, no such shell was found. However, the hybrid origin of the specimen presented in figure 1 is very likely. It is also not sure that the hybridization of the two species has always to lead to such heterosis. Hence, only an electrophoretical study on rich material from the Niepolomice Forest can give exact data on hybrid frequencies. Such a study is planned.

The observed interspecific differences in allozyme pattern, different alleles being found in at least one locus in almost any enzyme system studied, seem to indicate that *Viviparus contectus* and *V. viviparus* are very old species

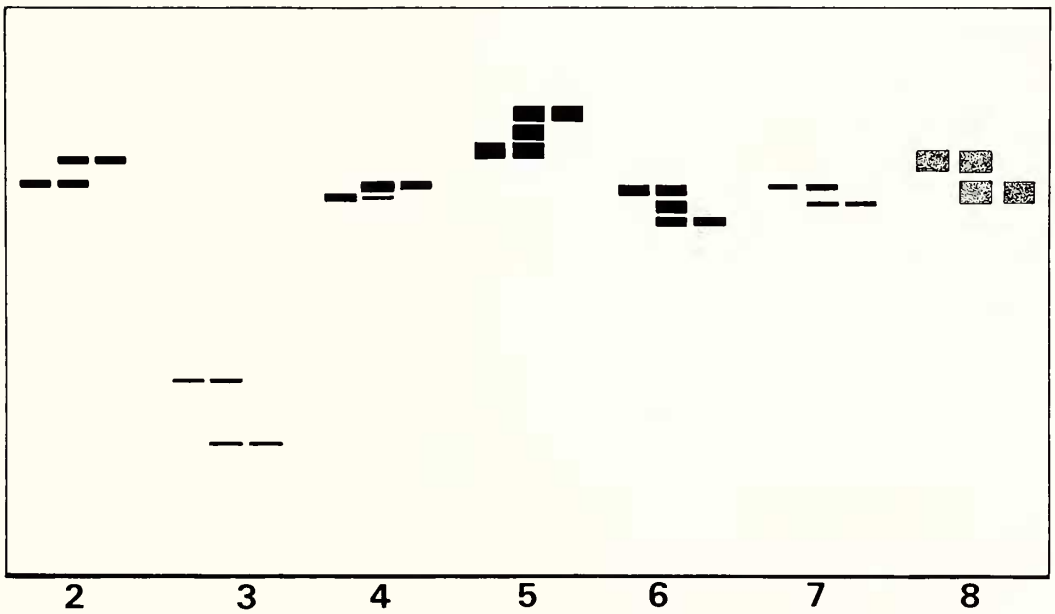

Fig. 4. Schematical diagram of isozyme electrophoretic patterns obseved in *Viviparus viviparus, V. contectus*, and the hybride individual Only these zones (presumable loci) are presented, which show no allele shared by the two species. For each enzyme system, the heterozygotic hybride specimen is in the middle, *V. viviparus* in the left, and *V. contectus* in the right. Numbers of enzyme systems as in Table 2 (2, the slower of two activity zones; 3, the fastest of several zones detected; 4, the faster of two zones; 8, one of several zones detected).

and the isolating mechanisms that prevent their hybridization could have broken down after such a long time since the speciation event (the case discussed by Wiley, 1981) which is the more probable, now that they occur sympatrically very rarely.

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